the block face is approximately 5 nm, the resolution along the z-axis in SBF-SEM is limited by the minimum slice thickness of around 25 nm. We have explored the feasibility of improving the z-resolution in SBF-SEM by recording images at more than one primary beam energy, thus sampling different depths below the block surface. We used Monte Carlo simulations of SEM images from an epoxy block containing 5-nm diameter carbon spheres stained with 14% osmium positioned at different depths, as a model for small biological structures. A linear relationship was found between the depth of the spheres and the ratio of backscattered signals at primary beam energies of 1.4 keV and 6.8 keV, which allowed us to generate 3D tomograms with a depth resolution of around 5 nm. Experiments are in progress to test this technique using a Zeiss Sigma-VP SEM equipped with a Gatan 3View SBF system. Sub-surface SBF-SEM could potentially match focused ion beam (FIB) SEM in terms of z-resolution, but with the added advantage of providing higher throughput and larger tissue volumes. The research was supported by the intramural program of NIBIB.

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Fixed Path Length Sample Holders Enable Robust Cryosaxs Measurements from Sub-Microliter Sample Volumes

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Small angle x-ray scattering (SAXS) gives structural information about biological molecules in solution. However, large (~30 microliter) sample volumes are needed to mitigate radiation damage, limiting the use of SAXS in studying rare molecules. By cryocooling SAXS samples, radiation damage and required sample volumes are reduced by orders of magnitude [1], but challenges in creating identically-sized frozen samples complicate background subtraction. Here we present microfabricated silicon sample holders for cryoSAXS. These rigid sample holders have a fixed x-ray path length, simplifying background subtraction. Less than 800 nL of sample are required, facilitating measurements on expensive or hard-to-express molecules. These fixed path length, low volume sample holders make cryoSAXS a more accessible technique capable of probing a wide range of biological molecules.

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3D Dynamical Observations of Single Molecule Motions by X-Rays, Electron and Neutron

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We have proposed that single molecule techniques using shorten wavelengths, for example, X-rays, electrons, and neutron [1]. Especially, Diffracted X-Ray Tracking (DXT) using normal synchrotron orbital radiation (SR) source (not XFEL) has been developed for obtaining the information of the 3D internal motions of single proteins with both high time-resolution (micro-seconds) and high precision (nm/1000) [2, 3]. DXT can be monitored through trajectories of the Laue diffraction spots from the nanocrystal which was labeled on the individual proteins. This concept can apply to utilize by using both electrons and neutron. Instead of the Laue diffraction using white X-ray, the Electron Back-Scattered Diffraction Pattern was adopted to monitor the 3D orientations of the nanocrystals linked to the single protein molecules[4]. We called Diffracted Electron Tracking (DET). Additionally, we call Diffracted Neutron Tracking (DNT) for new single molecule measuring method in which the long time observation from the non-destructivity of a neutron is possible.

DXT, DET and DNT are assigned to labeling techniques through the nanocrystals. The size effect between intramolecular motions of individual proteins and the labeled nanocrystals becomes very important. We succeeded in the analysis of the quantitive size effects. As a result, we pointed out a possibility that determinations of the intramolecular motions without labeled nanocrystals are carried out quantitatively. Additionally, by progressing of the automatic DXT analysis corresponding to huge diffraction information, we obtained the time-resolved dynamical information that statistical reliability is sufficiently high.

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Accurate Determination of Tautomeric/Protonation States in Quantum-Mechanic Driven Macromolecular Crystallographic Refinement

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Structure Based Drug Discovery (SBDD) is employed by virtually all pharmaceutical research and development organizations. Gaining an understanding of the protein:ligand complex structure along with the proper protonation and explicit solvent effects is crucial for obtaining meaningful results from docking, thermodynamic calculations, active site exploration, and ultimately lead optimization. Recently, we incorporated our linear-scaling, quantum mechanics (QM) DivCon tool with Phenix (e.g. Phenix/DivCon) in order to accurately elucidate the protein:ligand complex molecular structure. An intrinsic problem of the X-ray crystallographic data is its inability to detect hydrogen atoms - even at higher resolutions. It is generally extremely difficult to experimentally determine the protonation/tautomeric state of the ligand and the surrounding active site. Traditionally, protonation can be established using the neutron diffraction; however, experimental requirements such as reliance on very large crystals and on deuterium exchange limit the method's suitability in SBDD.

In order to address this X-day data deficiency, we have challenged Phenix/DivCon with various protonation candidates and applied rigorous statistical analyses to measure the agreement between the 3D structure of each candidate with electron density. While through the experiment we still cannot directly observe hydrogen atoms, using the accurate QM functional we are able to observe the presence/absence of hydrogen atoms by studying their influences on bound heavy atoms (Carbon, Nitrogen, Oxygen). To evaluate our protocol we have chosen two protein:ligand structures 4N9S and 2JJJ for which both neutron and X-ray structures and data are available in PDB. Ten probable protonation states for the ligands in those structures have been generated, and each of the possible candidates has been refined against X-ray data with Phenix/DivCon. We have found out that the top scored tautomer in each case coincides with the ligand structure revealed by the neutron diffraction.

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Transmission X-Ray Imaging Detector Captures the Last Light at NSLS

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Biological research constitutes a large and expanding scientific focus at synchrotron facilities. Structural biology researchers using x-ray facilities make up the majority of this community, including use of techniques such as macromolecular x-ray crystallography, small-angle x-ray solution scattering, x-ray microscopy, x-ray absorption spectroscopy, x-ray fluorescence and x-ray footprinting. Many of these technologies, as they are developed to take advantage of next-generation synchrotron sources, are trending toward use of high flux beams and/or beams which require enhanced stability and precise understanding of beam position and intensity from the front end of the beamline all the way to the sample. For high flux beams, major challenges include heat load management in optics (including the vacuum windows) and a mechanism of real-time volumetric measurement of beam properties such as flux, position, and morphology. For beam stability in these environments, feedback from such measurements directly to control systems for optical elements or to sample positioning stages would be invaluable. For x-ray footprinting, a focused ''white beam'' is used to maximize x-ray flux density over a practical sample size using a toroidal mirror. This intense beam can melt beryllium windows and is very complicated to measure, causing difficulties with properly focusing the mirror and with understanding where the beam is and exactly what is being delivered to the sample. To address these challenges, we are developing diamond-based instrumented vacuum windows with integrated volumetric x-ray intensity, beam profile and beam-position monitoring capabilities. The prototype device will be used as the exit window for the XFP beamline currently being developed at NSLS-II for x-ray footprinting. Current progress is presented, including successful demonstration of a >1kilopixel free-standing transmission imaging detector that was used to capture the last x-ray photons at the National Synchrotron Light Source.